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IN-VITRO EVALUATION OF NITRIC OXIDE SCAVENGING ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACT OF *SYZYGIUM CUMINI LINN*. BARK (MYRTACEAE)

Kuncha Jayachandra*[1], Ayyachamy Maheswaran [2], Mariappan Murali [3]

Department of Pharmaceutical Chemistry [1, 3], Department of Pharmaceutics [2], College of Pharmacy, Jaya College of Paramedical Sciences, Chennai-602024, Tamil Nadu, India

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Correspondence to Author:

Kuncha Jayachandra

Assistant Professor, Department of Pharmaceutical Chemistry, Jaya College of Paramedical Sciences College of Pharmacy, C.T.H Road, Thiruninravur, Chennai-602024, Tamil Nadu, India

ABSTRACT

Plants have provided mankind with herbal remedies for many diseases for many centuries and eventody. In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as Ayurveda, Unani and Sidha. *Syzygium cumini* Linn. (Myrtaceae) commonly known as jambul tree (English), having promising theraputic values with its various phytoconstituents. Preliminary Phytochemical investigation was carried out on the methanolic extract of *Syzygium cumini* Linn. Bark. It indices presence of Carbohydrates, Amino acids, Tannins, Saponins, phytosterols, Terpenoids, phenols and flavonoids. We are also quantitatively estimated total phenolic content, tannins and favaniods by using spectrophotometer. The total phenolic content was 580.23 ± 3.03 mg/g, tannin content was 534 ± 4.03 mg/ g while the flavonoid content was 315.42 ± 4.52 mg/g. The methanolic and aqueous extracts of bark were screened for antioxidant activity using Nitric oxide scavenging activity method, which showed significant persentage of inhibition in dose dependent manner. As antioxidant therapy is found to be useful in complicated disease status related with free radical activity. This is the first research report regarding Invitro evaluation of Nitric oxide scavenging activity of Methanolic and aqueous extracts of *Syzygium cumini* bark. The present study might be extended for the formulation and evaluation of different antioxidant herbal dosage forms.

INTRODUCTION: Free radicals have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane [1]. The body on the other hand, requires free radicals for immune responses. However, an overload of these molecules had been linked to certain chronic diseases of heart, liver and some form of cancers [2]. Atoms of oxygen or Nitrogen having central unpaired electron are called as reactive oxygen or Nitrogen species [3, 4]. The role of Nitric oxide (NO) in numerous disease state have generated a considerable discussion over the past several years since the Journal Science named it the molecule of the year in 1992. NO is important bioregulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial activity. Low concentration of NO, are sufficient in most cases to affect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects [5]. Experiments have demonstrated that NO plays a

catabolic role in the development of osteoartheritis and mediates the inflammatory response, it is also involved in the degradation of matrix metalloproteins, inhibits the synthesis of both collagen and proteoglycons [6]. The NO molecule is very unstable and reacts with oxygen produce intermediates such as NO_2, N_2O_4, N_3O_4. The stable products nitrate, Nitrite and peroxynitrite when treated with superoxide [7]. The molecular mechanism as to how NO enhance cancer development has become an active area of research. NO mediates S- nitrosylation of key enzymes and regulatory proteins plays critical role.

Key proteins S-nitrosylated which enhance tumor development include several caspases involved in apoptosis, PTEN the tumor suppressor protein, Bcl-2 the mitochondrial protein which protects from apoptosis, OGG1 the DNA repair protein and methinine adenosyl transferase the liver protein which synthesizes adenosylmethionine. The S-adenosylation of these proteins enhances DNA mutations, prevents cancer cell apoptosis and enhances oncogenic cell growth [8]. Problems with memory and social functions in patients with Schizophrenia may result from an imbalance in the brain's Nitric oxide system.

An experimental result shows that rate with characteristics of schizophrenia regain normal functions if they receive drugs that reduce the production of nitric oxide in the brain [9]. Herbal drugs have been used by mankind since time immemorial to treat various disorders and after an alternative to the synthetic compounds, as they have been considered either non-toxic or less toxic. The traditional Indian system of Medicine, Ayurveda is based on the principle of balance and counter balance. Ayurveda (Ayu= life, Veda=Knowledge) extensively uses the plant derived compound formulations for the treatment of various ailments after a careful study into the type of the disease [10].

Syzygium cumini belonging to the family of Myrtaceae is a large evergreen tree up to 30 meters height and girth of 3.6 meters with a bole up to 15 meters found throughout India up to an altitude of 1,800 meters. Most of plant parts of *Syzygium cumini* are used in traditional system of medicine in India. *Syzygium cumini* matured stem bark powder is light brown, shows fragments of thin-walled cork cells, aseptate fibers, single or in groups, oval to angular, elongated, stone cells; rosette and prismatic crystals of calcium oxalate and simple round to oval starch grains measuring 5-11 µ in diameter [11].

Previous phyto-chemical investigations on this species bark revealed the presence of butulinic acid, β-sitosterol, friedelin, epi-friedelanol [12]. It also contains new esters of epi-friedelanol (eugenin) [13], D-glucoside, kaempterol-3-O-glucoside, quercetin [14], myricetin, astragalin and gallic acid [15].

The bark is astringent; its juice is given (56-112 ml) doses in chronic diarrhoea, dysentery, menorrhagia. Decotion of the bark is an efficacious mouth-wash and gargle for trating spongy gums, stomatitis, relaxed throat and other diseases of mouth. Bark used for inflammation of skin [16], it is also used in dyeing and tanning and for coloring fishnets. Pharmacologically it can be used as anti-helmintic [17], and in good for sore throat, bronchitis, asthma, biliousness, and cure ulcers [18].

Even though, more research works has been carriedout on these plants, there is no sufficient scientific data available regarding Nitric oxide scavenging activity of *Syzygium cumini* bark.

Therefore, our aim in this study was to evaluate the antioxidant activity of methanolic and aqueous extracts of *Syzygium cumini* bark by Nitric oxide scavenging activity method.

MATERIALS AND METHODS:

Plant Material: The fully mature, fresh stem bark of *Syzygyum cumini* was collected from Midhilanagaram, Mellacheruvu village, Chittoor district, AndhraPradesh. The stem bark was identified and authenticated by Dr. S. B. Narasimha Reddy, Professor, Department of Botany, S.G. Govt. Degree College, Piler and voucher specimen (No.- JCP/2010/153) was deposited in the Herbarium of the same department. The bark was air dried at room temperature (25^{o}C) for 30 days and converted into fine powder with an automix blender; the powder was kept in a deep freezer until the time of use.

Preparation of Extracts: 500 gm of dry fine powder was suspended in 1.5 liters of methanol and double distil water separately then stirred magnetically for 24 hours at room temperature. The extracts were double filtered by using muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40^{o}C using rotary vacuum evaporator (Buchi labortech AG, Switzerland) to obtain crude extract. The dried MESCB and AESCB (Methanolic & Aqueous extracts of *Syzygyum cumini*) were stored in vacuum desiccators under controlled conditions till it used for experimental purpose.

Drugs and Chemicals used: The Sodium Nitroprusside (SNP), Griess reagent, Ascorbic acid and Methanol (95% V/V) were obtained from S.D. Fine Chemicals, Mumbai. All the chemicals and solvents were of analytical grade.

Preliminary Phytochemical Screening: 1 gm of the methanol extract of *syzygyum cumini* bark were dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (w/v). The standard methodology of Harborne (1998) [19] and Kokate (2001) [20] were adopted for the phytochemical screening.

Determination of Total Phenolic content, Tannins and flavonoids: The total phenolic content in the extracts were determined using Folin-ciocalteau reagent accoding to the Malic and Singh (1980) [21]. Tannin content was determined by Folin-Denis reagent according to the method of Schandrel (1970) using tannic acid as standard [22]. The favonoids were estimated by earlier reported method (Ivan *et al*., 2004) [23].

Nitric Oxide generation and assay of Nitric Oxide scavenging method: Nitric Oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO [24, 25] which interact with molecular oxygen to produce Nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO [26]. Sodium Nitroprusside (10 mM) in phosphate buffer saline (PBS) was mixed with different concentrations of extract (100-1000 µg/ml) of the drug dissolved in ethanol and water and incubated at 25^{o}C for 180 minutes.

The samples from above were reacted with Griess reagent (1% Sulphanilamide, 0.1% Napthylenediamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with Napthylenediamine dichloride was read at 546 nm and referred to the absorbance of ascorbic acid, used as a positive control treated in the same way with Griess reagent [27].

Nitric Oxide scavenged (%) = $A_{Control} - A_{Test} / A_{Control} \times 100$

Where, $A_{Control}$ = Absorbance of control reaction; A_{Test} = Absorbance in the presence of the samples of extracts.

RESULT AND DISCUSSION: Preliminary phytochemical analysis showed the presence of Phenols, Terpenoids, Tannins, Saponins, Phytosterols, Carbohydrates, Flavonoids, Amino acids like phytoconstituents (**Table-1**) may be responsible to show a potent antioxidant activity. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl group [28].

TABLE 1: PRILIMINARY PHYTOCHEMICAL SCREENING OF METHANOLIC AND AQUEOUS EXTRACTS OF *SYZYGIUM CUMINI* BARK

Phytochemicals	Methanolic & Aqueous extracts
Alkaloids	-
Amino acids	+
Anthraquinones	-
Flavonoids	+
Carbohydrates	+
Phytosterols	+
Saponins	+
Tannins	+
Terpenoids	+
Phenols	+

+ Presence, - Absence

The phenolic content may contribute directly to the antioxidant activity [29]. It has been suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [30]. Consequently, the antioxidant activity of methanolic extract is often explained by their total phenolic content, tannins and flavonoids contents with good correlation.

The total phenolic content in the methanolic extract of *Syzygium cumini* was 580.23 ± 3.03 mg/g, tannin content was 534 ± 4.03 mg/ g while the flavonoids content was 315.42 ± 4.52 mg/g. These results demonstrate that tannins represent the main group of phenolic compounds in *Syzygium cumini* bark [31].

Evaluation of Nitric Oxide Scavenging Activity: Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is diffusible free radicals that play many roles as an effector molecules diverse biological system including neuronal messenger, vasodilatation, antimicrobial and anti tumor activities [32]. Suppression of released NO may be partially attributed direct NO scavenging, as the extracts of *Syzygium cumuni* decreased the amount of nirite generated from the decomposition of SNP *in-vitro*.

The scavenging of NO by the extracts was increased in dose dependant manner. **Figure 1** illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The methanol and aqueous extracts showed maximum activity of 74% and 67% respectively at 1000 µg/ml where as ascorbic acid was 84.06% at the same concentration. The IC_{50} values were found to be 800 µg/ml, 900 µg/ml for methanolic and aqueous extracts and 600 µg/ml for ascorbic acid respectively. The given results are diverted to methanolic and aqueous extracts of *Syzygium cumini* is having NO scavenging activity but less than ascorbic acid.

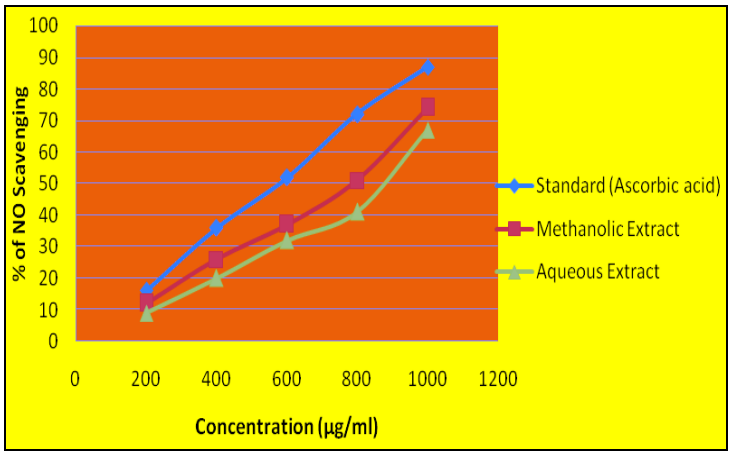

FIG. 1: NITRIC OXIDE (NO) SCAVENGING ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF *SYZYGIUM CUMINI* BARK

CONCLUSION: The methanolic and aqueous extracts of *Syzygium cumini* bark exhibited significant antioxidant activity. The results are compared with ascorbic acid and the activity may be related to the phenolic contents and flavonoids in this plant extract. Since reactive oxygen species are important contributors to several serious ailments.

In the present study, the observed NO scavenging activity of the methanolic and aqueous extracts of *Syzygium cumini* bark might be useful for the development of newer and more potent natural antioxidants.

Furthermore, detailed studies on isolation, characterization of phytochemicals, Pharma-cological and biochemical investigation is needed to elucidate the mechanism of action and will helpful in projecting this *Syzygium cumini* bark as a theraputic targent in antioxidant reserach.

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